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Selected Heamatological Parameters of Broiler Birds Fed Low-Protein Diets Supplemented With the Most Limiting Essential Amino Acids

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Abstract: *This study is theheamatologicalevaluation of the possibility of augmenting the low-crude protein broiler diets with adequate supplementation with the most limiting essential amino acids (EAAs), L-Lysine, DL-Methionine, L-Tryptophan and L-Threonine. Two sets of experimental diets were prepared for the two phases of broiler production at broiler starter (BS) and broiler finisher (BF) phases. At the BS phase (0-28 days), crude protein levels were varied from 23.0% in both the control diet (conventional BS diet with plant protein origin and fish meal) and diet 2 with the most limiting EAAs to diet 6 with the lowest crude protein of 11.0%. At the BF phase (29-57 days), six dietary treatments in which crude protein varied from 20.0% to 8.0% were used. Except for lymphocytes, most of the heamatological parameters investigated such as heamoglobin concentration (Hbc), packed cell volume (PCV), red blood cell (RBC), erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell heamoglobin (MCH), mean cell haemoglobin concentration (MCHC), neutrophils, monocytes, basophils and eosinophils all had similar values (P>0.05) among their treatment means. However, slight insignificant variations exist among the mean values of most parameters with a range between 6.68±2.74g/100ml (for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation) and 9.78±0.97g/100ml for Hbc. The mean values for PCV vary from 20.50±10.66% to 29.50±4.04%. The RBC ranged from the lowest value of 1.35±68.87 ($10^6/mm^3$) to 2.23±38.98 ($10^6/mm^3$). The ESR ranged from 2.25±0.96 (mm^3/l) to 7.25±5.91(mm^3/l). MCV ranged slightly from 0.11±0.02 ($\times10^{-6}$ul)to 0.14±0.01 ($\times10^{-6}$ul). MCH ranged from the lowest value of 4.19±0.41 ($\times10^{-6}$ug) to 4.93±0.22 ($\times10^{-6}$ug). MCHC ranged from the lowest value of 32.40±0.21 to 41.8±0.12 Lymphocytes varied significantly (P<0.05) from the lowest value of 59.0±0.82 to 62.25±0.05. The neutrophils ranged from the lowest value of 22.25±0.96 to 26.25±2.99. Monocytes ranged from the lowest value of 11.50±2.52 to 13.0±2.58. Basophils had almost the same average values for birds across all diets. Eosinophils also had similar (P>0.05) values.The adequate essential amino acid supplementation had possibly obviated the immunodepressive tendency of the low-crude protein diets on the broiler chickens.*

Keywords: *Immunodepression, lymphocytes, most limiting essential amino acids*

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I. Introduction

A deficiency of dietary protein or amino acids has long been known to impair immune function and increase the susceptibility of animals and humans to infectious diseases [1]. The underlying cellular and molecular mechanisms began to unfold only in the past 15 years with the revelation that protein malnutrition reduces concentrations of most amino acids in plasma. Findings from recent studies indicate an important role for amino acids in immune responses by regulating: (1) the activation of T lymphocytes, B lymphocytes, natural killer cells and macrophages; (2) cellular redox state, gene expression and lymphocyte proliferation; and (3) the production of antibodies, cytokines and other cytotoxic substances[1].

Increasing evidence shows that dietary supplementation of specific amino acids to animals and humans with malnutrition and infectious disease enhances the immune status, thereby reducing morbidity and mortality. However, care must observed to correct possible negative impact of imbalance and antagonism among amino acids on nutrient intake and utilization in order to develop effective strategies of enteral or parenteral provision for maximum health benefits.

The crude protein level is reported as a predisposing factor for necrotic enteritis [2, 3, 4, 5].Moreover, an excess of protein would lead to physiological need for an increase in water consumption to achieve efficient nitrogen excretion. As a consequence, high crude protein diets lead to higher level of nitrogen and water excretion compared with low crude protein diets, leading to deterioration in litter quality and the bird's environment [6].Because leucocytes are important targets for the actions of amino acids, here webriefly review the pertinent literature related to the defense against infectious diseases.

The immune system protects the host from various pathogens and consists of the innate (natural, non-specific) and the acquired (adaptive, specific) systems [7].It is likely that nutrients influence several or all aspects of the immune system. Thus, there are multiple, complex methods for assessing immune function in individuals, depending on experimental conditions, the availability of analytical facilities and the investigator's interest [8, 9].

The study is therefore conducted to ascertain the possibility of augmenting the low-protein broiler diets with the most limiting essential amino acids supplementation without significantly affecting the immune system of the broiler birds.

II. Materials And Methods

2.1 Experimental Site

The experiment was carried out in the Poultry Unit of the Teaching and Research Farm (T & RF) of Ekiti State University, Ado-Ekiti with geographical coordinates of 7^o 38'0" North, 5^o 13' 0" East The T &RF and a tropical humid climate with distinct wet and dry seasons. The rainy season spans over seven months starting from March/early April to October with a dry spell in August. Temperature in this area is fairly uniform throughout the year with little deviation from the mean annual of 27^{o}c. The topography is moderately sloppy with the highest point having the slope of not greater than 6%. The main vegetation is grass but activities like bush fallowing influences vegetation. The experiment was carried out between March and June, 2016. Further laboratory analyses were carried out at the Animal Production and Health Sciences Laboratories of Ekiti State University and The Federal University of Technology, Akure.

2.2 Site Preparation

Prior to the arrival of broiler chicks, the poultry house and metabolism cage were thoroughly washed and fumigated with diskol (a disinfectant containing 4% benzalkonium chloride, 3% glutaraldehyde, 14% formaldehyde, stabilizers, antioxidants and activators). The house was covered to prevent heat loss and brooding equipment installed.

2.3 Sourcing of Pharmaceutical Feed-Grade Amino Acids

Feed-grade L-Lysine, L-Tryptophan and L-Threonine amino acids were ordered from Ajinomoto Animal Nutrition, Ajinomoto North America, Inc., 4020 Ajinomoto Drive, Raleigh, USA. Pharmaceutical-grade amino acids are reputed to be between 99% and 100% pure. Ajinomoto amino acids are used for multiple nutritional applications, including intravenous solutions, infant and pediatric formulas and other dietary supplements. They are on the list of U.S. Food and Drug Administration (FDA) having complied with the current Good Manufacturing Practice (cGMP). Ajinomoto is a research leader in the nutritional physiology of amino acids, generating knowledge and understanding of the properties and behavior of amino acids, and creating even more medical and nutritional applications. DL-Methionine was purchased locally from a reputable feed mill in Akure, Ondo State, Nigeria

The technical information on the feed grade amino acids are as follows:

L-Lysine Monohydrochloride (feed grade, 78.8%)

Commercial Guarantee:

L-Lysine content	78.8% minimum
Physical data:	
Chemical formula	$C_6H_{14}N_2O_2HCL$
Molecular weight$_1$	182.65
Appearance	Yellowish-white, crystalline powder
Purity (L-lysine base)	78.8% (on as-is basis); 80.0% (dry matter basis)
Dry matter	98.5% (minimum)
Bulky Density	0.55 to 0.65grams/cm^3 (34.3 to 40.5 lbs/ft^3)
Total nitrogen$_1$	15.12% (on an as-is basis)
Chloride (Cl)	19.40% (on an as-is basis)

Crude protein (% 16g-N_1)	94.40% (on an as-is basis)	
Calculated energy		
Poultry, ME	4,120 (kcal/kg)	1870 (kcal/lb)
Stability:		
Shelf life	3 years (25 kg Bags); 1year (1,000 kg Totes)	
Pelleting stability	stable under commercial pelleting conditions	

L-Threonine (feed grade, 78.8%)

Commercial Guarantee:	
L-Threonine content	98.5% minimum
Physical Data:	
Chemical formula	$C_4H_9NO_3$
Molecular weight$_1$	119.12
Appearance	white to off- white crystalline or granule
Content	98.0% (on an as-is basis); 98.5% (dry matter basis)
Dry matter	99.5% (minimum)
Bulk Density	0.6 to 0.7 grams/cm^3 (37.4 to 43.7 lbs/ft^3)
Total nitrigen$_1$	11.52% (on an-is basis)
Crude protein (%/16g-N_1)	72.03% (on an as-is basis)
Calculated energy:	
Poultry, ME	3,570 (kcal/kg) 1,619(kcal/lb)
Stability:	
Shelf life (unopened packaging)	3 years (25 kg Bags); 1year (1,000 kg Totes)
Pelleting stability	stable under commercial pelleting conditions

L-Tryptophan (feed grade, 98.0% on a dry matter basis)

Commercial Guarantee:	
L-Tryptophan content	98.0% (on a dry matter basis)
Physical data:	
Chemical formula	$C_{11}H_{12}N_2O_2$
Percent Nitrogen	13.45% (on an as-is basis)
Molecular weight$_1$	204.23
Appearance	Yellowish-white, crystalline powder
Solubility in H_2O	1.136 g/100 g water at 20ºC
Purity	98.0% (Min)
Dry matter	98.5% (minimum)
Bulky Density	0.20 to 0.30grams/cm^3
Chloride (Cl)	19.40% (on an as-is basis)
Crude protein (% 16g-N)	84.0% (on a dry matter basis)
Poultry, ME	5,850 (kcal/kg) or 2,650(kcal/lb)
Stability:	
Shelf life	3 years (25 kg Bags); 1year (1,000 kg Totes)
Pelleting stability	stable under commercial pelleting conditions

DL-Methionine (feed grade, 99.0% on a dry matter basis)

Commercial Guarantee:	
DL-Methionine content	99.0% minimum
Physical Data:	
Chemical formula	$C_5H_{11}NO_2S$
Molecular weight$_1$	149.21
Appearance	white to light grey crystal or powder
Content	99.0% (dry matter basis)
Dry matter	99.0% (minimum)
Bulk Density	0.55 to 0.65 grams/cm^3

2.4 Experimental Diets

The feed ingredients used in ration formulation were purchased locally from a reputable commercial feed miller. Feed-grade amino acids were sourced as previously discussed. The experimental diets were compounded and manually mixed on the clean floor of the Poultry Section of the Teaching & Research Farm. The dietary treatments were made up of the control diet 1 which had an approximate value of 20.0% crude protein of both plant and animal (fish meal) origins with a substantial supplementation of DL-Methionine and L-Lysine. Diet 2 also had 20.0% all of plant origins with four most limiting EAAs. Diets 3, 4, 5 and 6 contained reduced inclusion levels of crude protein of plant origin at approximate values of 17.0%, 14.0%, 11.0% and 8.0%, respectively. In essence, crude protein was reduced by 3 points across the diets from diet 3 to diet 6. The four most limiting essential amino acids [10] in broilers were supplemented as required in the low crude protein diets.

Table 1. Requirement for crude protein and the most rate limiting amino acids for broilers [10]

	Weeks of Age		
Nutrients, %	**0 -3**	**3-6**	**6-8**
Crude protein	23.00	20.00	18.00
Methionine	0.59	0.38	0.32
Total sulfur			
Amino acids	0.90	0.72	0.60
Lysine	1.10	1.00	0.85
Threonine	0.80	0.74	0.68
Tryptophan	0.20	0.18	0.16
Isoleucine	-.80	0.73	0.62
Arginine	1.25	1.10	1.00
Valine	0.90	0.82	0.70

Table 2. Experimental diets for chicks fed varying crude protein levels with amino acids supplementation at broiler starter phase (1 – 28days)

INGREDIENTS	**Control Diet 1**	**Crude Protein Reduction/Amino Acids supplementation**				
		Diet 2	**Diet3**	**Diet 4**	**Diet 5**	**Diet 6**
		23.0% CP	**20.0% CP**	**17.0% CP**	**14.0% CP**	**11.0% CP**
Maize (11% CP)	56.4	56.7	56.7	56.7	56.7	56.7
Soyabean meal (45% CP)	36.0	39.0	32.0	25.0	18.0	11.0
Fish meal (72% CP)	3.0	-	-	-	-	-
Rice husk	-	-	5.0	5.0	11.0	16.0
Palm oil	-	-	2.0	5.0	6.0	8.0
Bone meal	2.5	2.5	2.5	2.5	2.5	2.5
Oyster shell	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.3	0.3
*Premix	0.5	0.5	0.5	0.5	0.5	0.5
Amino acid supplementation						
Lysine	0.15	1.5	1.5	1.5	1.5	1.5
Methionine	0.15	1.0	1.0	1.0	1.0	1.0
Threonine	-	1.0	1.0	1.0	1.0	1.0
Trytophan	-	0.5	0.5	0.5	0.5	0.5
Calculated composition						
Crude Protein, %	23.3	23.0	19.8	16.6	13.7	10.8
**ME (Kcal/Kg)	2893.3	2889.7	2884.0	2960.1	2872.5	2866.8
Crude Fibre, %	3.9	4.1	3.8	3.4	7.4	9.0
Fat, %	3.8	3.6	3.5	3.3	3.1	2.9
Proximate Composition						
Crude Protein	23.1	23.3	18.9	16.9	14.2	11.3
Crude Fibre, %	3.9	4.1	3.8	3.4	7.4	9.0
Fat, %	3.8	3.6	3.5	3.3	3.1	2.9
MC, %	9.35±0.08	9.42±0.04	9.46±0.06	9.43±0.03	9.36±0.06	9.34±0.05

Contained vitamins A(10,000,000iu); D(2,000,000iu); E(35,000iu);K(1,900mg); B12 (19mg); Riboflavin(7,000mg); Pyridoxine(3,800mg); Thiamine(2,200mg); D Panthotenic acid(11,000mg); Nicotinic acid(45,000mg); Folic acid(1,400mg); Biotin (113mg); and trace elements as Cu(8,000mg); Mn(64,000mg); Zn(40,000mg); Fe(32,000mg); Se(160mg);* I_2*(800mg); and other items as Co(400mg); Choline(475,000mg); Methionine(50,000mg); BHT(5,000mg) and Spiramycin(5,000mg) per 2.5kg CP:Crude Protein, ME:Metabolized Energy.ME, metabolizable energy = (0.860+0.629(GE-0.78CF) [11].*

Table 3. Experimental diets for broilers fed varying crude protein levels with EAA supplementation (finisher phase, 29 – 57days)

INGREDIENTS	Control Diet 1	Crude Protein Reduction/Amino Acids supplementation				
		Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
		20.0% CP	17.0% CP	14.0% CP	11.0% CP	8.0% CP
Maize	65.4	61.1	56.8	57.3	57.1	59.1
Soyabean meal	28.0	32.0	25.3	19.8	13.0	6.0
Fish meal (72% CP)	2.0	-	-	-	-	-
Rice husk	-	-	8.0	10.0	15.0	20.0
Palm oil	-	-	3.0	6.0	8.0	8.0
Bone meal	2.5	2.5	2.5	2.5	2.5	2.5
Oyster shell	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.3	0.3
*Premix	0.5	0.5	0.5	0.5	0.5	0.5
EAA supplementation						
Lysine	0.15	1.0	1.0	1.0	1.0	1.0
Methionine	0.15	0.7	0.7	0.7	0.7	0.7
Threonine	-	0.7	0.7	0.7	0.7	0.7
Trytophan	-	0.2	0.2	0.2	0.2	0.2
Calculated composition						
Crude Protein, %	19.9	19.9	17.0	14.1	11.0	7.7
**ME (Kcal/Kg)	2893.3	2889.7	2807.16	2890.74	2871.32	2828.23
Crude Fibre, %	3.9	4.1	3.8	3.4	7.4	9.0
Fat, %	3.8	3.6	3.5	3.3	3.1	2.9

**Contained vitamins A(10,000,000iu); D(2,000,000iu); E(35,000iu);K(1,900mg); B12 (19mg); Riboflavin(7,000mg); Pyridoxine(3,800mg); Thiamine(2,200mg); D Panthotenic acid(11,000mg); Nicotinic acid(45,000mg); Folic acid(1,400mg); Biotin (113mg); and trace elements as Cu(8,000mg); Mn(64,000mg); Zn(40,000mg); Fe(32,000mg); Se(160mg); I_2(800mg); and other items as Co(400mg); Choline(475,000mg); Methionine(50,000mg); BHT(5,000mg) and Spiramycin(5,000mg) per 2.5kg;CP:Crude Protein, ME:Metabolized Energy.*

***ME, metabolizable energy = (0.860+0.629(GE-0.78CF) [11]*

2.5 Management of Experimental Birds

Two sets of three hundred broiler chicks were collected from reputable hatchery and two hundred and eighty eight were randomly picked after their sexes were determined. The chicks were brooded in a brooder house using electricity supplied constantly by 1KVA stand-by power generating plant at the Ekiti State University Teaching and Research Farms. During the first week of the broiler starter phase, the chicks were fed on commercial chicks mash containing 23% crude protein (CP) before the commencement of the experiment. The chicks were managed on the floor for this phase of experiment.

Appropriate veterinary routines were observed from day old.

2.6 Experimental Design

Two hundred and eighty eight (288) broilers chicks were randomly assigned into 6 experimental treatments using a completely randomized design (CRD). The 6 treatments were replicated 4 times and each replicate contained 12 birds. The average weights of birds in each replicate were taken and carefully balanced to ensure uniformity of weights in all treatments.

2.7 Blood Collection for Analyses

At the end of the feeding trials of the two broiler phases, all birds will be starved overnight. One male chick from each replicate will be randomly picked and blood will be collected through the wing-web vein using a 2ml syringe and needle. Blood samples will be put in labeled bijour bottles containing a speck of EDTA. The bottles will be covered and content mixed by inversion. The blood samples collected will be used for haematological studies. The packed cell volume (PCV%) will be estimated in heparinized capillary tubes in anhaematocrit micro centrifuge for 5minutes with 1400 RPM. Total red blood cell (RBC) count will be determined using Drabkin solution to easily recognize red blood cells from other components of the blood under microscope. The haemoglobin concentration (HBC) will be estimated, whereas mean corpuscular heamoglobin concentration (MCHC) and mean corpuscular volume (MCV) will be calculated.

2.8 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) and means among treatments were separated accordingly using Computer Minitab Statistical Package (Version 16).

III. Results And Discussion

The haematologicaland biochemical serum parameters investigated are presented in Tables 4 and 5.

All biochemical serum parameters investigated had similar (P>0.05) values for birds on all diets with slight insignificant (P>0.05) variation across the diets.

Except for lymphocytes, most of the haematological parameters investigated such as haemoglobin concentration (Hbc), packed cell volume (PCV), red blood cell (RBC), erythrocyte sedimentation rate (ESR), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), neutrophils, monocytes, basophils and eosinophils all had similar values (P>0.05) among their treatment means. However, slight insignificant variations exist among the mean values of most parameters with a range between 6.68±2.74g/100ml (for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation) and 9.78±0.97g/100ml for Hbc. The mean values for PCV vary from 20.50±10.66% in birds on diet 3 (17.0% CP diet with EAAs supplementation) to 29.50±4.04% for birds on diet 6 (8.0% CP diet with EAAs supplementation).

The RBC ranged from the lowest value of 1.35±68.87 ($10^6/mm^3$) for birds on the control diet (conventional diet with animal protein and minimum methionine and lysine supplementation) to 2.23±38.98 ($10^6/mm^3$) for birds on the 8.0% CP diet with EAAs supplementation. The ESR ranged from 2.25±0.96 (mm^3/l) for birds on diet 6 (8.0% CP with EAAs supplementation) to 7.25±5.91(mm^3/l) for birds on diet 3 (17.0% CP with EAAs supplementation). MCV ranged slightly from 0.11±0.02 ($\times10^{-6}$ul) in birds on diet 3 (17.0% CP with EAAs supplementation) to 0.14±0.01 ($\times10^{-6}$ul) and 0.14±0.02 ($\times10^{-6}$ul) in birds on diets 4 (14.0% CP with EAAs supplementation) and diet 6 (8.0% CP with EAAs supplementation), respectively. MCH ranged from the lowest value of 4.19±0.41 ($\times10^{-6}$ug) for birds on diet 6 (8.0% CP with EAAs supplementation) to 4.93±0.22 ($\times10^{-6}$ug) for birds on the control diet 1 (conventional control diet with animal protein and minimum methionine and lysine supplementation).

MCHC ranged from the lowest value of 32.40±0.21 for birds on diet 3 (17.0% CP with EAAs supplementation) to the highest value of 41.8±0.12 for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation. Lymphocytes varied significantly (P<0.05) from the lowest value of 59.0±0.82 for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation to 62.25±0.05 for birds on diets 5 (11.0% CP with EAAs supplementation) and 6(8.0% CP with EAAs supplementation). The neutrophils ranged from the lowest value of 22.25±0.96 for birds on diet 6 (8.0% CP with EAAs supplementation) to 26.25±2.99 for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation. Monocytes ranged from the lowest value of 11.50±2.52 for birds on diet 3 (17.0% CP with EAAs supplementation) to the highest value of 13.0±2.58 for birds on diet 2 (20.0% CP with EAAs supplementation). Basophils had almost the same average values for birds across all diets with a slight insignificant variation (P>0.05) ranging from the lowest value of 2.00 for birds on diet 4 (14.0% CP with EAAs supplementation) to the highest value of 2.50 for birds on the conventional control diet with animal protein and minimum methionine and lysine supplementation. Eosinophils had the least but similar (P>0.05) values of 0.75±0.50 for birds on diet 2 (20.0% CP with EAAs supplementation) and diet 4 (14.0% CP with EAAs supplementation) to the highest value of 1.0 for birds on other diets.

The result in the present study disagreed with previous studies who posited that in addition to improving growth performance with pigs and broilers, Threonine supplementation of diets enhanced immune functions as measured by antibody responses to different antigens [12, 13, 14]. However, it has been indicated that reducing dietary CP content up to 3 percentage points impaired the immunocompetence of broiler chicks with a conclusion that dietary CP and individual amino acids have important roles in immune function [13, 14].

The present work has a support in previous work [15]where cellular and humoral immunity in broilers fed diets supplemented with Thrwere evaluated and there was no observation of any improvement in immunocompetence. Also, in a more recent work [16], it was reported that the reduction of dietary crude protein content tended to decrease primary (p=0.16) and secondary (p=0.13) immune responses against sheep red blood cell

(SRBC) but there were some evidences that Thr modulates immune functions [1, 17, 18] and that the immune system is sensitive to dietary Thr intake [19].

Another previous study [20], it was reported that a decrease in dietary crude protein and arginine (Arg) levels diminished the antibody production response to Newcastle disease virus and posited that the broken-line analysis indicate that Arg requirements of starting broiler chicks for optimal immune functions (107% of NRC values) are higher than those for maximum growth performance (101%) or feed efficiency (103%) and are dependent on dietary protein concentration.It was also reported that reducing dietary protein content from 22.35 to 19% in broiler starter diet was associated with a decrease ($P < 0.001$) in peripheral blood lymphocyte mitogenesis [20]. Conversely, when there was an increase in dietary CP level from 19 to 22.35%, there was an increase ($P<0.001$) in the proportion of lymphocytes and consequently lower ($P<0.05$) heterophil-to-lymphocyte ratio.

Table 4. Heamatological indices of matured broiler birds fed varying crudeprotein levels with amino acids supplementation

Parameters	Control Diet 1 20.0% CP	Crude Protein Reduction/Amino Acids supplementation				
		Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
		20.0% CP	17.0% CP	14.0% CP	11.0% CP	8.0% CP
Hbc, g/100ml	6.68±2.74	7.93±1.42	6.83±3.58	7.68±0.97	7.58±0.51	9.78±0.97
PCV,%	21.75±8.88	23.75±4.35	20.50±10.66	23.00±2.94	22.75±1.50	29.50±4.04
RBC,10^6/mm^3	1.35±68.87	1.65±54.57	1.73±55.16	1.44±34.73	1.43±19.77	2.23±38.98
ESR, mm^3/l	7.00±4.16	4.00±2.30	7.25±5.91	4.50±1.73	4.75±0.96	2.25±0.96
MCV ×10^{-6} (ul)	0.12±0.01	0.13±0.02	0.11±0.02	0.14±0.01	0.13±0.03	0.14±0.02
MCH ×10^{-6} (ug)	4.93±0.22	4.77±0.23	4.41±0.31	4.61±0.22	4.32±0.23	4.19±0.41
MCHC	41.8±0.12	32.60±0.31	32.40±0.21	34.10±0.24	32.84±0.21	36.16±0.31
Lymphocytes	59.00±0.82^{c}	60.50±0.58^{bc}	61.00±0.82^{ab}	62.00±0.82^{ab}	62.25±0.50^{a}	62.25±0.50^{a}
Neutrophils	26.25±2.99	23.50±2.65	24.25±3.10	22.75±1.71	22.50±2.38	22.25±0.96
Monocytes	11.75±2.50	13.00±2.58	11.50±2.52	12.50±2.08	12.00±2.45	12.75±0.96
Basophils	2.50±0.58	2.25±0.50	2.25±0.50	2.00±0.00	2.25±0.50	2.25±0.50
Eosinophils	1.00±0.00	0.75±0.50	1.00±0.00	0.75±0.50	1.00±0.00	1.00±0.00

a, b, c, Means within a row with different superscript are significantly different (P<0.05).
Hbc, haemoglobin concentration; PCV, packed cell volume; RBC, red blood cell; ESR, erythrocyte sedimentation rate;
MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration

Table 5. Some biochemical serum indices of matured broiler birds fed varying crudeprotein levels with amino acids supplementation

Parameters	Control Diet 1 20.0% CP	Crude Protein Reduction/Amino Acids supplementation				
		Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
		20.0% CP	17.0% CP	14.0% CP	11.0% CP	8.0% CP
Total Protein	31.53±0.43	27.06±0.45	27.54±0.63	28.26±	27.01±	20.02±
Albumin	25.33±	23.47±	23.92±	24.91±	22.98±	17.52±
Globulin	6.2±	3.59±	3.62±	3.35±	4.03±	2.5±
Alb/Glo	4.09	6.54	6.61	7.44	5.70	7.01
ALP (IU/I)	214.7±0.54	215.4±0.62	209±0.45	213±0.76	214±0.86	215±.93
CHOL (mg/dl)	44.2±0.43	51.1±0.48	45.9±0.76	52.0±0.54	51.1±0.34	
ALT (IU/I)	5.3±0.34	5.5±0.56	5.8±0.65	6.1±0.45	5.9±0.43	6.0±0.54
AST (IU/I)	10.0±0.56	10.1±0.43	10.2±0.67	10.0±0.68	10.2±0.45	10.0±0.56

a, b, c, Means within a row with different superscript are significantly different (P<0.05).
Alb/Glo, albumin globulin ratio; ALP, alanine phosphatase; CHOL, cholesterol;
ALT, alanine aminotransferase; AST, aspartate transaminase

IV. Conclusion

The similarities in values obtained for most haematological and serum parameters in all experimental diets including diets in which CP were significantly reduced with essential amino acids supplementation were indicative of the fact that there was no sign of immunodepression.

The adequate essential amino acid supplementation had possibly obviated the immunodepressive tendency of the low-crude protein diets on the broiler chickens.

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